ACTA CHROMATOGRAPHICA, NO. 14, 2004

SELECTIVE SEPARATION AND DETERMINATION OF CEPHALOSPORINS BY TLC ON STANNIC OXIDE LAYERS

*S. A. Nabi**, *E. Laiq*, and *A. Islam*

Analytical Research Laboratory, Department of Chemistry, Aligarh Muslim University, Aligarh-202002, India

SUMMARY

The chromatographic behaviour of some cephalosporins has been studied on synthetic inorganic ion-exchanger (stannic oxide) layers using citrate and borate buffers as mobile phases. Several ternary and quaternary separations have been achieved. The utility of these separations has been demonstrated for estimation of cephalosporins in blood serum from patients.

INTRODUCTION

The use of inorganic ion exchangers as adsorbents in thin layer chromatography has afforded promising results in the separation of metal ions, anions [1–4], organic compounds [5,6], phenols [7] and organic acids [8]. The widespread application of ion-exchange resins for separating and purifying amino acids from protein hydrolysis has received considerable attention [9,10]. Stannic oxide has been used for TLC because it has been found to be quite stable in acids, bases, and common organic solvents; it has been used for separation of metal ions [11], anions [12], pesticides [13], and phenolic compounds [14].

Addition of inorganic salts to conventional adsorbents and use of organic and inorganic solvents as mobile phases has been reported to result in improved separations of some pharmaceutical products compared with the untreated adsorbent [15]. Double hydroxide adsorbent layers have been used for TLC separation of cephalosporins [16]. The cephalosporin antibiotics are a large family of therapeutically useful compounds. The basic nucleus of the cephalosporins is 7-aminocephalosporanic acid (7-ACA) which comprises a dihydrothiazine nucleus and a β-lactam ring (Fig. 1).

Substitution of the various R_1 and R_2 groups results in cephalosporins with different pharmacological and pharmacokinetic properties [17].

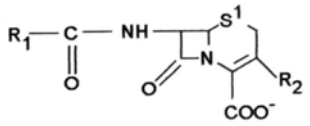

Fig. 1

The cephem nucleus

Sporadic publications on the identification of cephalosporin antibiotics by densitometry on hydrocarbon-impregnated silica gel HPTLC plates have appeared in the literature [18–20] and TLC of cephalosporins has also been performed on silanized silica gel [22,23]. Recovery and purification of streptomycin antibiotics have been conducted successfully on an industrial scale [23]. In the work reported here the utility of stannic oxide and simple mobile phases for separation of cephalosporins has been studied for the first time. The TLC procedure described enables simple and rapid separation and detection of different spontaneous, chemical, and enzymatic degradation products of the cephalosporins. Suitable combination of mobile phase and spray/detection reagent enables identification of these products in aqueous preparations and in biological fluids and microbiological culture broths.

EXPERIMENTAL

Chemicals

Methanol (AR grade), citric acid, sodium hydroxide, potassium dihydrogen phosphate, ammonia solution, borax, sodium bicarbonate, ferric ammonium sulphate, and hydroxylamine hydrochloride were from Merck (Mumbai, India). Boric acid was from Ranbaxy (S.A.S. Nagar, India). Nickel(II) chloride hexahydrate was from Qualigens Fine Chemical (Mumbai, India).

Samples

Cefaclor was from Aristo pharmaceuticals (Nani Daman, Mumbai, India), ceftriaxone from Otomotive Products (Navi Mumbai, India), ceftazidime from Biochem Pharmaceuticals (Mumbai, India), cefoperazone from

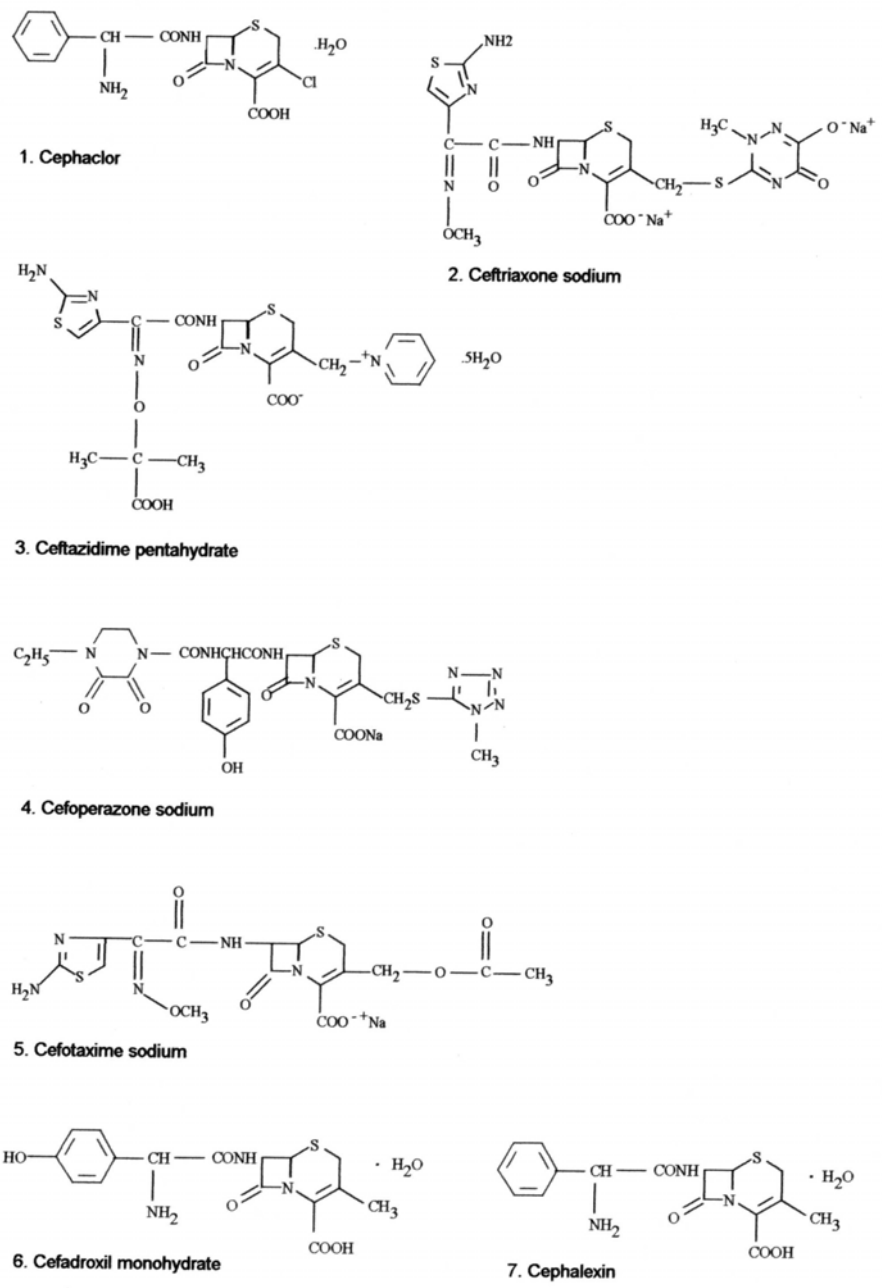

Fig. 2

The molecular structures of the cephalosporins studied

Unimed Technologies (Halol, Gujarat, India), cefotaxime from Starry Health Care (Vikhroli (W) Mumbai, India), cefadroxil from Comed Chemicals (Baroda, Gujarat, India), and cephalexin from Glaxo India (Mumbai, India). The structures of the compounds are shown in Fig. 2.

Stationary Phase

The inorganic ion exchanger (stannic oxide) was prepared by a method reported elsewhere [13]. The TLC plates were prepared in the usual manner from a demineralized water (DMW) slurry, dried in laboratory air overnight, and activated at 60°C for 1 h before spot application.

Mobile Phase

At least 20 different solvents were used as mobile phases but separation of cephalosporins on stannic oxide was achieved solely by use of citrate and borate buffers of different pH. The mobile phases used are listed in Table I.

Table I

The mobile phases used

Components	pH	Abbreviation
0.2 M Boric acid	1.25	S1
Citrate buffer (0.2 M citric acid + 0.2 M sodium hydroxide)	3.15	S2
Citrate buffer (0.2 M citric acid + 0.2 M sodium hydroxide)	4.05	S3
Citrate buffer (0.2 M citric acid + 0.2 M sodium hydroxide)	5.05	S4
Borate buffer (0.2 M boric acid + 0.2 M sodium hydroxide)	9.10	S5
Borax	9.18	S6
Borate buffer (0.2 M boric acid + 0.2 M sodium hydroxide)	10.03	S7
Aqueous ammonia (30%)	13.55	S8
0.1 M Potassium dihydrogen phosphate	5.30	S9

Chromatography

For qualitative studies solutions (1.0 mg $mL^{-1)}$ of each cephalosporin in DMW were applied at one end of the plate. The plates (7.0 in × 7.0 in) were developed, at room temperature (20 ± 2°C), in rectangular chambers (20 cm × 22 cm × 9 cm) previously equilibrated by conditioning with mobile phase for at least 1 h. The time required for chromatographic development of the plates varied with the mobile phase used. After development plates were dried with a stream of cold air and the spots were visualized

by placing the plates in a chamber of iodine vapour. Brown spots on the plate revealed the location of the compounds. hR_F values were calculated by means of the formula:

$$hR_f = \frac{\text{distance travelled by the geometrical centre of the solute spot}}{\text{distance travelled by solvent front from the point of application}} \times 100$$

The hR_F values obtained are listed in Table II.

Table II

hR_f values of the cephalosporins on stannic oxide layers developed with different mobile phases

No.	Cephalosporin	S1	S2	S3	S4	S5	S6	S7	S8	S9
1	Cephalexin	87.50	72.22	11.22	77.27	94.44	84.61	87.60	42.42	45.00
2	Cefadroxil	42.85	72.22	0.00	77.27	43.75	15.30	37.50	62.85	57.14
3	Cefaclor	100.00	87.21	76.47	85.00	100.00	100.00	97.77	71.42	50.00
4	Cefotaxime	85.71	0.00	76.47	0.00	87.50	92.30	75.00	73.33	65.38
5	Ceftriaxone	0.00	0.00	0.00	0.00	0.00	0.00	0.00	62.50	15.38
6	Ceftazidime	0.00	0.00	0.00	0.00	45.00	100.00	31.25	74.50	70.00
7	Cefoperazone	57.14	0.00	0.00	31.25	43.75	38.45	43.75	75.75	71.00

Quantitative Separations

For quantitative work, stock solutions of cephalosporins were prepared in DMW. Solutions of different cephalosporins were mixed, spotted by means of a microsyringe, and developed with a selected mobile phase. A pilot plate was run simultaneously to facilitate exact positioning of the spot on the working plate. The regions containing the cephalosporins were scraped from the plates, added to DMW, then filtered. The clear solution containing the cephalosporin content of each spot was then analysed by a standard spectrophotometric method [24–28]. Results are shown in Table III.

Quantitative Estimation of Cephalosporins in Blood Serum Samples

Anhydrous sodium sulphate (30 g) and ethanol (95%, 20 mL) were mixed with 10 mL of oxalate blood. The mixture was centrifuged and after 2 h the supernatant liquid was decanted. Because of the presence of sodium sulphate and alcohol, water was eliminated; this was an essential step ensuring correct estimation. Sodium sulphate also prevents emulsification which might occur as a result of the presence of hexose sugars. Finally the mixture

Table III

Quantitative separations of cephalosporins from synthetic mixtures on stannic oxide layers

No.	Separations achieved	Amount taken (μg)	Amount found (μg)[a]	Recovery (%)	Error (%)	*SD*[a]	Mobile phase[b]
1	Cefaclor	50.00	49.45	98.90	–1.10	0.018	S2
	Ceftriaxone	50.00	50.05	100.10	0.10	0.070	
	Cefadroxil	50.00	49.65	99.30	–0.70	0.007	
2	Ceftriaxone	50.00	50.25	100.10	0.10	0.079	S4
	Cefoperazone	50.00	50.01	100.02	0.02	0.048	
	Cephalexin	50.00	50.12	100.24	0.24	0.062	
3	Ceftazidime	50.00	49.67	99.34	–0.66	0.090	S1
	Cefotaxime	50.00	49.76	99.52	–0.48	0.065	
	Cefadroxil	50.00	49.58	99.16	–0.84	0.097	
4	Cefotaxime	50.00	49.80	99.60	–0.40	0.014	S9
	Cefadroxil	50.00	49.64	99.28	–0.72	0.013	
	Ceftriaxone	50.00	50.14	100.28	0.28	0.008	
5	Cefaclor	50.00	49.45	98.90	–1.10	0.017	S3
	Ceftriaxone	50.00	50.05	100.10	0.02	0.083	
	Cephalexin	50.00	50.13	100.26	0.26	0.021	
6	Cefaclor	50.00	49.45	98.90	–1.10	0.017	S6
	Ceftriaxone	50.00	50.05	100.10	0.02	0.070	
	Cefoperazone	50.00	49.87	99.74	–0.26	0.168	
	Cefadroxil	50.00	49.58	99.16	–0.84	0.097	
7	Ceftriaxone	50.00	50.05	100.10	–0.84	0.079	S5
	Ceftazidime	50.00	49.68	99.36	0.64	0.183	
	Cefotaxime	50.00	49.80	99.60	–0.40	0.014	
	Cephalexin	50.00	49.64	99.28	–0.72	0.013	

[a]Average from five replicate determinations

[b]S1, Boric acid (pH 1.25); S2, citrate buffer (pH 3.15); S3, citrate buffer (pH 4.05); S4, citrate buffer (pH 5.05); S5, borate buffer (pH 9.10); S6, borax buffer (pH 9.18); S9, potassium dihydrogen phosphate (pH 5.30)

was shaken with diethyl ether for 30 min. The ether layer was separated and the mixture was reduced to approximately 0.05 mL under vacuum at 40–45°C. Blood samples (serum) from different patients were first analysed for specific antibiotics by a standard spectrophotometric method [24–27]. A known amount of the concentrated solution was then applied to the TLC plates and the plates were developed with an appropriate mobile phase. The region containing the spot was scraped from the plate, mixed with DMW, and same procedure was applied as in quantitative separation.

RESULTS AND DISICUSSION

Antibiotics are chemically defined reproducible chemical substances produced in and isolated from living cells, or are chemical or biological derivatives of these [29]. Non-specific methods for analysis of antibiotics, for example microbiological and spectrophotometric methods, do not differentiate between structurally similar by-products from the synthesis or degradation of the antibiotics of interest. More specific methods such as TLC, GC, and HPLC that differentiate among different structures are preferable for analysis of antibiotics [30]. Because of the unstable nature of antibiotics, decomposition of the drugs or polymerization can occur during chromatography, although this occurs less in TLC than in paper chromatography [31].

Stannic oxide is regarded as quite stable, amorphous, and hydrated to a variable extent. TLC plates are, therefore, activated at 60°C to desorb physically bonded water. The spots were detected by placing the TLC plates in an iodine vapour chamber. The iodine vapour dissolves in or forms weak charge-transfer complexes with organic compounds and the cephalosporins show up as brown spots on a pale yellow background within few minutes. After marking the zones for further reference exposure of the plates to air causes the iodine to sublime and the spots fade. The hR_F values of the different cephalosporins after chromatography with mobile phases S1 to S9 are shown in Table II. It is clear from the hR_F values that cefaclor, which contains a Cl group, has higher hR_F values for most of the mobile phases than cephalexin (OCH_3 group) or cefadroxil (CH_3 group). This might be because of the negative inductive effect – Cl^- has a stronger negative inductive effect than OCH_3 (i.e. attracts electrons more strongly).

It was found that the behaviour of cefoperazone was peculiar in almost all of the mobile phases except ammonia and potassium dihydrogen phosphate. The positive inductive effects of the methyl and ethyl groups in cefoperazone reduce chemical interaction with the mobile phase and hence migration is suppressed. On the other hand, the presence of the Na^+ ion in cefotaxime, ceftazidime, and cefoperazone facilitates release of an electron, leading to the formation of polar compounds that are highly soluble in water and, therefore, of low R_F in acidic media and high R_F value in basic media, as expected.

It is apparent from Table II that separation of most of the cephalosporins is poor with the ammonia mobile phase, probably because of solvation of the alkali metals by the ammonia molecule. The exceptional

behaviour of ceftriaxone chromatographed with ammonia and potassium dihydrogen phosphate enables selective separation of this antibiotic from the other cephalosporins. On the basis of the different R_F values of the cephalosporins when chromatographed with different mobile phases it is possible to achieve some important binary and ternary quantitative separations on stannic oxide layers in a few minutes only. It is worth noting one advantage of using stannic oxide layers – the possibility of selective separation of some components of a synthetic mixture of cephalosporins. The results are reported in Table III.

This TLC method is simple, rapid, selective, reproducible, and applicable to identification and separation of cephalosporins. The practical utility of this method was demonstrated by quantitative identification of common cephalosporins in serum samples from patients .The results are summarized in Table IV. The percentage recovery, accuracy, and reproducibility of the method were checked statistically.

Table IV

Quantitative separations of cephalosporins from samples of patients' serum

Serum sample no.	Cephalosporin	Amount taken (μg)	Amount found (μg)[a]	Recovery (%)	*SD*[a]	Mobile phase[b]
1	Cefaclor	28.00	27.62	98.64	0.05	S 1
2	Cefotaxime	90.00	88.52	98.35	0.125	S 6
3	Ceftriaxone	151.00	150.63	99.75	0.038	S 8
4	Ceftazidime	43.00	42.79	99.51	0.022	S 6

[a]Average from four replicate determinations
[b]S1, boric acid (pH 1.25); S6, borax buffer (pH 9.18); S8, aqueous ammonia (pH 13.55)

ACKNOWLEDGEMENT

The authors are grateful to Chairman, Department of Chemistry, Aligarh Muslim University, Aligarh, for providing the necessary research facilities.

REFERENCES

[1] S.D. Sharma, T.D. Sharma, and B.C. Sethi, J. Liq. Chromatogr., **6**, 1253 (1983)
[2] N.S. Sethi, R.P.S. Rajput, and N.K. Agarwal, Anal. Lett., **18**, 481 (1985)
[3] S.A. Nabi, W.U. Farooqui, and N. Rahman, J. Planar Chromatogr., **7**, 38 (1994)
[4] G. Vanik and S.W. Hussain, Anal. Sci., **16**, 1079 (2000)
[5] S.A. Nabi, W.U. Farooqui, and Z.M. Siddiqui, J. Liq. Chromatogr., **6**, 109 (1983)
[6] S.A. Nabi, W.U. Farooqui, and N. Rahman, Chromatographia, **20**, 109 (1985)
[7] K. Sahil, S.K. Dabral, and K.P.S. Muktawat, J. Indian Chem. Soc., **79**(9), 739 (2002)
[8] K. Sahil, S.K. Dabral, and K.P.S. Muktawat, J. Indian Chem. Soc., **78**, 374 (2001)
[9] L. Lepri, P.G. Desideri, and D. Heimler, J. Chromatogr., **268**, 493 (1983)
[10] S.D. Sharma, H. Sharma, and S.C. Sharma, Chem. Environ. Res., **11**, 179 (2002)
[11] N. Zattrezie-Renault, J. Inorg. Nucl. Chem., **40**, 539 (1978)
[12] A.K. Sen and U.C. Ghosh, J. Liq. Chromatogr., **3**, 31 (1980)
[13] S.A. Nabi, A. Gupta, M.A. Khan, and A. Islam, Acta Chromatogr., **12**, 201 (2002)
[14] A. Gupta, PhD thesis, Department of Chemistry, Aligarh Muslim University, Aligarh, India, 1999, pp. 98–114
[15] J.A. Manthey and M.E. Amundson, J. Chromatogr., **19**, 522 (1965)
[16] S.Z. Qureshi, R.M.A.Q. Jamhour, and N. Rahman, J. Planar Chromatogr., **9**, 466 (1996)
[17] K. Venkatraman (ed.), Biogard's Cephalosporins Hand Guide, Bangalore-560 086, India, 1999, p. 4
[18] S.C. Dhanesar, J. Planar Chromatogr., **11**, 195 (1998)
[19] S.C. Dhanesar, J. Planar Chromatogr., **11**, 258 (1998)
[20] S.C. Dhanesar, J. Planar Chromatogr., **12**, 114 (1999)
[21] J. Hoogmartens and E. Roets, J. Assoc. Off. Anal. Chem., **46**, 173 (1981)
[22] I. Quintes, J. Eykens, E. Roets, and J. Hoogmartens, J. Planar Chromatogr., **6**, 181 (1993)

[23] J. Sherma and B. Fried (eds), Handbook of Thin-Layer Chromatography, Marcel Dekker, New York, 1990
[24] D.L. Mays, F.K. Bangert, W.C. Cantrell, and W.G. Evans, Anal. Chem., **47**, 2229 (1975)
[25] F.I. Sengun and Koksal Ulas, Talanta, **33**, 363 (1986)
[26] F.I. Sengun and I. Fedai, Talanta, **33**, 366 (1986)
[27] P.B. Issopoulos, J. Pharm. Biomed. Anal., **7**, 619 (1989)
[28] British Pharmacopeia, Vol. 1, 1980, p. 86
[29] R. Brunner, in R. Brunner and G. Machek (eds), Die Antibiotica, Hans Carls, Nuremberg, FRG, (1962), p. 7
[30] J. Ripphahn and H. Halpaap, J. Chromatogr., **112**, 7 (1975)
[31] E.J. Vandamme and J.P. Voets, J. Chromatogr., **71**, 141 (1972)